THE NATURAL HISTORY OF *CUMINGIA TELLINOIDES.*

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INTRODUCTION.

When it is considered that *Cumingia* is used extensively as a source of material for embryological and experimental studies it becomes a matter of importance to know its life history and its availability at all times. An observation made several years ago indicated that *Cumingia* exhibits a lunar periodicity in spawning and led the writer to make an extended study of the species covering a continuous period of five years. The laboratory work was carried on at the Marine Biological Laboratory at Woods Hole.

With the perfecting of the diatom method of feeding embryos it is possible to carry many marine larvæ through metamorphosis and thereby gain a knowledge of complete life histories where only fragmentary knowledge has been possible before. In this instance a pure culture of the diatom Nitschia, which was isolated at the Plymouth Laboratory, was used.[2] The diatoms at Woods Hole are not suitable for feeding because of the danger of bacterial infection of the embryos. The Beaufort species first used by Caswell Grave, on the other hand, grows in great abundance in aquarium jars and may be fed without difficulty. This diatom has the advantage over Nitschia in being relatively short and thick instead of long and slender. Both of these species should be propagated at marine laboratories for the use of investigators.

HABITAT.

Cumingia tellinoides is found principally in sandy bottoms containing a strong admixture of humus, with a sparse or moderate growth of eel grass, and where the tidal currents run swiftly as in narrow channels. The vertical distribution is not great, being a little above and below low tide mark.

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[1] From the Osborn Zoölogical Laboratory, Yale University, New Haven, Conn., and the Marine Biological Laboratory, Woods Hole, Massachusetts.

[2] The writer wishes to thank Dr. Isabella Gordon for a stock of this material.

The animal is readily dug with a spade at low tide and separated from the dirt with a coarse sieve. Under favorable conditions one can collect three or four dozen in the course of an hour. It has long siphons which enable it to lie inches below the surface of the bottom and yet feed from the water in the manner of lamellibranch mollusks generally, the food being chiefly diatoms and floating particles of organic matter. It is rather particular about the kind of bottom and the nature of the water currents and is therefore restricted in its distribution.

The Spawning Season.

Cumingia begins to spawn about the middle of June and continues until the middle of September. One can usually depend upon getting eggs in abundance until about August 20. At that time there is likely to be a lull or often a complete cessation in spawning until the first of September or until the next full moon. Eggs can usually be obtained in considerable quantity during the first half of September. Not all individuals are spawning in September and few are spawning heavily as a rule. Each adult spawns more than once during the season and the production of gametes is apparently continuous.

Method of Securing Eggs.

Cumingia spawn very readily in the laboratory but it is necessary to treat them according to schedule. Experience has shown that the best way to obtain clean eggs or sperm is to wash the animals free from all sediment and isolate them in small stender dishes half filled with sea water. When so placed in sea water and left undisturbed for an hour or less they extend their siphons and spawn if sexually mature. The eggs or sperm are thrown forcibly from the dorsal siphon as they accumulate in the supra-branchial chambers. The sexes are separate and may be distinguished by their color. The males are white and the females pink. The shell is not heavy so that the color of the gonads shows through more or less.

Cumingia cannot be kept in the laboratory indefinitely. It is desirable to use them the day they are collected, although it is sometimes possible to keep them for a day or two in moist sand. They must be kept away from water if it is desirable to have them retain their eggs or sperm.

THE EGG. SIZE, STRUCTURE, MEMBRANES.

The egg of *Cumingia* measures in extreme limits from .061 mm. to .065 mm. while the usual size is .062 or .063 mm. In color it varies from a light gray to a distinct pink. The animal pole of the egg is comparatively free from yolk and pigment so that it appears light in color. Centrifuged eggs show a beautiful banding due to the separation of materials of different specific gravity. These materials consist of yolk, pigment, oil and clear protoplasm.

The polarity of the egg is fairly rigidly fixed as shown by the fact that in centrifuged eggs the cleavage planes remain unchanged although the pigment and so-called formative materials may be driven to any part of the egg. This general problem was investigated by Morgan in 1910 and the phase of it referred to here was fully verified by myself in 1924. He was concerned with the influence of the "organ-forming substances" upon development.

Surrounding the egg there is a jelly membrane about .03 mm. in thickness or half the diameter of the egg. It is wholly invisible under the microscope but readily demonstrable by the use of a suspension of india ink in the water. One might suspect the presence of this jelly membrane by the circumstance that the eggs in a dish do not come into contact but are separated by a little space. This tertiary membrane has apparently been overlooked by investigators. There is also a distinct vitelline membrane which undergoes little or no visible change after fertilization. The membrane is not perceptibly lifted after the penetration of the spermatozoön so it may be proper to say that there is no fertilization membrane. This may account for the fact that the *Cumingia* egg is at the mercy of the sperm and polyspermy is common. At any rate it is desirable to learn whether this susceptibility to polyspermy is due to absence of one or more of the egg secretions that Lillie has shown to be concerned with the fertilization reaction in the Nereis egg, and which sweep supernumerary spermatozoa from the surface of the egg after the initiation of the fertilization reaction. It frequently happens that one can count ten or a dozen sperm nuclei within an egg that has been too heavily inseminated. Cases have been seen in which every egg showed polyspermy of this extreme character. When eggs are spawned the germinal vesicle has already broken down and the first polar spindle has formed. On this account the first polar body appears very promptly after insemination.

Morgan found that the egg of *Cumingia* is very easily injured by rough handling so that it may fail to develop normally beyond cleavage. At first he was unable to secure normal veliger larvæ from centrifuged eggs. He finally centrifuged the entire animal before it had spawned and, to his surprise, it spawned completely centrifuged eggs which developed normally, showing that the dislocation of the egg materials did not interfere with normal development. He came to the conclusion that the trouble he had experienced was due to injury to the membranes of the egg.

Rate of Growth, Metamorphosis and Life History.

The polar body is of interest chiefly because of its great size and mode of formation. It seems to round up before it comes to the surface of the egg and to migrate out, breaking its way through the cytoplasm of the egg as the latter is pushed up into a rounded prominence. The vitelline membrane, being thick and elastic, is pushed up locally. As a curious anomaly, no doubt associated with polyspermy, one case was observed in which two polar bodies came off at the same time, side by side, and were followed after the usual interval by a third polar body. As a rule only two polar bodies are formed. The first one rarely divides, although its division has been observed now and then. In case of polyspermy the cell may go into the three-celled condition at the first cleavage, and the polyspermic embryos may be normal or abnormal. *Cumingia* would, in fact, be first class material for a study such as Boveri's on the Echinoderm egg.

The rate of development and age at metamorphosis are shown in Table I. The later stages were obtained by feeding the larvæ, by means of which they were kept alive for a month. One can distinguish the new parts of the shell, as added to the original larval shell, and so tell where new growth has occurred. It is quite likely that the development of the larvæ in the open ocean is more rapid and uniform than in the laboratory. However, the veligers seemed to thrive upon diatoms in a large aquarium jar kept agitated by a mechanical device, and in time a considerable number went through metamorphosis.

TABLE I.

SCHEDULE OF NORMAL DEVELOPMENT AT MEDIUM SUMMER TEMPERATURES, 20°–21° C.

First polar body	12 to 15 minutes
Second polar body	28 to 35 "
First cleavage	60 to 70 "
Second cleavage	90 to 102 "
Blastula	5 to 6 hours
Gastrula	7 to 10 "
Trochophore	12 to 18 "
Metamorphosing trochophore	20 to 30 "
Veliger complete	2 to 24 days
Metamorphosis of veliger into adult form	16 to 24 "
Sexually mature in one year	
Fully grown in four years	
Duration of life two to four years	

The measurements given in Table III. show that the veligers grew somewhat before metamorphosis but more rapidly afterwards. This is not fully in line with data on the rate of growth of *Chiton* (unpublished), which show that there is no measurable growth until after metamorphosis. *Chiton* seems to be unusual in this respect. The larvæ of *Cumingia* did not develop uniformly in this aquarium habitat. Some grew symmetrically, adding equally to the shell all around, and others grew asymmetrically. I cannot, therefore, be sure of the normal form. Moreover, there is very considerable variation in the proportions of the normal veligers of a single female. If variation is as extensive in all lamellibranchs as in *Cumingia* it is difficult to see how the early larvæ of different species can ever be distinguished satisfactorily by measurements, although Stafford has been able to recognize the older larvæ. The chief difference that I have observed between the young veligers of various lamellibranchs is in their general appearance. Some have a thicker shell than others and therefore refract light differently. As is well known the veligers of all lamellibranchs are so much alike that they can be distinguished, if at all, only by experts. The differences are largely indefinable and are apparent only after long study.

Table II.

Schedule of Development at Higher Summer Temperatures, 22°–23° C.

First polar body	7 to 9	minutes
Second polar body	27 to 30	"
First cleavage	45 to 50	"
Second cleavage	70 to 75	"

Eggs developing at the rate shown in Table II. may reach the complete veliger stage in from twenty-four to thirty hours. The rate of development which Morgan gives for *Cumingia* trochophores and veligers is considerably too slow, and his statement that unfertilized centrifuged eggs are intact after forty-eight hours is also erroneous. Unfertilized eggs fragment within twenty-four hours. It should be noted, however, that he was not particularly concerned with the rate of development and these oversights do not affect the validity of the conclusions he draws.

Table III.

Measurements at Various Ages.

Egg .061 to .065 mm.—average .062 to .063.
Blastula same as above.
Trochophore same as above but slightly elongated anteroposteriorly.
Two day veliger .074 x .090 x .055 mm. to .08 x .091 x .055 mm.
Ten day veliger .09 x .10 mm.
Two weeks veliger .095 x .105 mm.
Three weeks Cumingia metamorphosed .09 x .105 mm. to .097 x .155 mm.
Four to five months (size attained before first winter.), 6 x 5 mm. to 10 x 8 mm.
One year, 11 x 8 mm. to 13 x 9 mm. (sexually mature).
Two years, 14 x 11 mm. to 16 x 11 mm.
Three years, 17 x 12 mm. to 17 x 13 mm.
Four years, 18 x 12 mm. to 19.5 x 14 mm. (Largest specimens found).

BIOLOGICAL LABORATORY

Experiments are in progress to learn the normal length of life of *Cumingia*. It appears that they are approximately half

RATE OF DEVELOPMENT AT VARIOUS TEMPERATURES.

Date.	Temp.	1st Polar Body.	2d Polar Body.	1st Cleavage.	2d Cleavage.
Aug. 20	18° C.	12–15 min.	40–45 min.	100–107 min.	150–160 min.
Aug. 20	18° C.	13–16 min.	50–55 min.	105–115 min.	154–165 min.
Aug. 21 (Two lots)	20° C.	*A* 13 min. *B* 12 min.	33 min. 32 min.	71 min. 68 min.	103 min. 101 min.
Aug. 20 (Two lots)	20° C.	*A* 10–13 min. *B* 11–13 min.	30–32 min. 31–33 min.	68–72 min. 69–73 min.	101–105 min. 102–105 min.
Aug. 18	20° C.	13–15 min.	33–35 min.	67–70 min.	100–105 min.
Aug. 10	22° C.	8 min.	28 min.	48 min.	73 min.
Aug. 10	22° C.	10 min.	33 min.	50 min.	75 min.
Aug. 10	22° C.	8 min.	28 min.	48 min.	72 min.
Aug. 18	29° C.	7–8 min.	20–22 min.	42–45 min.	62–67 min.
Aug. 21	30° C.	7–8 min.	19–20 min.	35–37 min.	54–57 min.
Aug. 28	33° C.	5 min.	20 min.	38–41 min.	58–61 min.
Aug. 20	35° C.	Tried three lots of eggs. None developed successfully. A few put off polar bodies and a few cleaved irregularly: one or two cleavages only.			

Note: These data show a rapid increase in rate of development from 18° up to 22° C. They do not show a proportionate increase in rate above that. 33° C. shows injury and irregularity. Eggs refused to cleave at 35° C.

It is only fair to say that this table should not be used for the calculation of temperature coefficients because the temperature was not kept rigidly uniform during the course of the experiments. It is sufficiently accurate for the purposes of this paper. It is possible to recognize end points to the minute although not all eggs cleave at the same instant as is indicated here.

grown and sexually mature in one year. I have the impression that they do not survive many years. Rings of growth on the shell show that they usually survive from two to four years, but the amount of growth after the third year is negligible.

In addition to the summarized data on the rate of development given in Tables I. and II. which are averages, I give the record of actual experiments in Table IV. It will be noted that the rates of development of several lots of eggs at temperatures ranging from 18° to 35° C. are given. It is interesting that the increase in rate of development from 18° to 23° C. is much greater than it is from 23° to 30° C.

The summer temperatures of the water at Woods Hole range from 18° to 22° C., the lower temperatures being those of June and late September, the higher being attained in July and August. The optimum temperature for the development of the *Cumingia* egg appears to be around 25° C. although the egg never experiences this temperature in nature.

The difference in rate of development of embryos between 18° and 25° is truly remarkable. One therefore does not speak of the normal rate of development of an embryo without considering the temperature. Because of certain interesting features of this table the writer expects later to describe more in detail the temperature coefficient of development of cleaving eggs.

Polyspermy.

Eggs spawned according to the method here described are convenient for study because they can be artificially fertilized at will. It is important to use only a drop of concentrated sperm suspension to fertilize a small stender dish of eggs, (in about 40 cc. of water) otherwise polyspermy results and abnormal development follows. As stated above, the *Cumingia* egg is particularly susceptible to polyspermy and it might be a legitimate problem for students of fertilization to undertake to explain why this is so, giving due consideration to Lillie's suggestion, *Jour. Exp. Zoöl.*, Vol. 14, 1913.

The Longevity of the Gametes.

If *Cumingia* eggs are allowed to remain unfertilized they ultimately die and disintegrate, though it has been found by experiment that they vary greatly in their longevity. The eggs of some individuals are much more vigorous and survive for longer periods than those of others. The poorest lots of eggs fragment in from six to nine hours, and the best lots remain apparently normal from fifteen to twenty-two hours. The average lot of eggs begins to fragment at the end of nine or ten hours and 90 per cent. of them have fragmented after fifteen hours. We may say therefore that the average longevity of the unfertilized egg, during which normal fertilization is possible, is 10 to 12 hours. When using eggs older than these one may look for a considerable percentage of abnormal embryos. It is only the best lots of eggs which give normal development of all embryos when more than ten or twelve hours old although early cleavage may appear normal.

The average life of the sperm in the most dilute suspensions capable of giving 100 per cent. fertilization (1/6000 to 1/8000%) is 4 to 5 hours. The extremes are 3½ to 18 hours. If fresh eggs are added to these dilute sperm suspensions after nine or ten hours from forty to fifty per cent. of fertilizations often occur. On the average one can expect only 20 to 40 per cent. of fertilizations after nine hours and sometimes none at all. After ten hours they may fertilize from one to ten per cent. of the eggs. It was noted that abundant normal larvae developed from eggs that were fertilized by these aging spermatozoa up to nine or ten hours.

A sperm suspension of 1/500 to 1/750 % lives longer than the more dilute suspensions mentioned above. They may give 100% fertilization up to 9 or 10 hours and a considerable percentage of the spermatozoa may live for 12 hours. It was observed that they usually fertilize from one to five per cent. of the eggs from fifteen to twenty five hours. It is therefore apparent that the conditions existing in a concentrated sperm suspension are not the same as in more dilute suspensions.

The evidence goes to show that half of the spermatozoa that are shed into sea water under natural conditions die within five or six hours and that nine tenths of them die within ten hours. After a few hours most of the spermatozoa stop swimming and lie quietly except for a few lashings of the tail at intervals. In the presence of eggs they become greatly activated and again swim vigorously. A very few are capable of becoming so activated up to the time when the life of the suspension becomes extinct, and a few fertilizations result, (1 to 5 per cent.).

It is interesting to note that J. Nelson observed that oyster sperm in the presence of egg secretions would wear out their limited store of energy within an hour and become incapable of causing fertilization.

I have not observed that spermatozoa in very dilute suspensions quickly lose their power to fertilize eggs while still motile as described by Professor Lillie for *Nereis* sperm, but it was observed that in very dense suspensions spermatozoa retain their vitality and ability to fertilize from two to three days. This phenomenon has been observed also by Gemmil, Lillie and Cohen. Gemmil ascribes it to the nutritive quality of the spermatic fluid, while Lillie and Cohen show that "it is due, at least in part, to the inhibiting effect of CO_2 in solution or to hydrogen ion concentration." Of course this extreme condition is never met with in nature.

Behavior of the Larva.

The larva becomes ciliated as a late blastula and, as an early gastrula it develops a strong prototroch and rises to the top. It swims vigorously until the trochophore fully transforms to the veliger. After becoming a typical veliger it soon settles to the bottom temporarily and from that time on it swims less and less frequently until it approaches the time of metamorphosis. Late veligers rarely swim and the velum appears to become more and more an accessory feeding organ. A certain amount of feeble movement is maintained until metamorphosis is completed, and the velum disappears. The velum persists until the form of the larva indicates that metamorphosis is practically complete though it becomes less and less vigorous after the first few days of active swimming.

Lunar Periodicity.

As indicated by the foregoing statements *Cumingia* shows a lunar rhythm in spawning. The period of the first quarter is the period of restricted spawning and full moon until new moon or until nearly first quarter is the period of free spawning. During the summer of 1922 *Cumingia* was not spawning from August 15 until September 1. On September 1 spawning revived and eggs were obtained in abundance for several days following. (September 5 was full moon in 1922.) The cessation in mid-August may be described as sudden and the recovery in September was equally striking. This complete break was observed by others who were experimenting with *Cumingia* eggs. Records of other years show that such a clear-cut periodicity has been observed before but is not demonstrable every year. There is, however, almost without exception, a reduced spawning at the time of the first quarter of the moon not only in August but in June and July as well. There can be little doubt, therefore, that we are dealing here with a true lunar periodicity. There is always a period of from one to five days during the season when eggs are not obtainable after which spawning is revised.

SUMMARY.

The breeding season extends from the second week in June to the middle of September. The gametes are produced continuously and spawning occurs more than once. A rhythm is present which is interpreted to be a lunar periodicity.

The rate of embryonic development is variable but under usual conditions the larva metamorphoses in about three weeks. Growth during the first two years is comparatively rapid and average adult size is attained at the end of the second year. Fortunate individuals may continue to live and grow for four years. Sexual maturity is reached in one year and spawning is abundant although greater quantities of gametes are produced during the second year.

The egg of *Cumingia* is subject to polyspermy and abnormal development often occurs if too heavily inseminated. The unfertilized egg will retain its vitality for approximately nine to twelve hours during which it is capable of developing normally. The spermatozoa when set free in sea water usually die within four to nine hours, a few die within three hours and a few live for twelve or fifteen hours.

The *Cumingia* egg is favorable material for experimental study in that it is obtainable in abundance, is readily fertilizable artificially, and it withstands laboratory manipulation without injury.

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